Journal of Medical Virology 51:234–241 (1997)

Analysis of Influenza A H3N2 Strains Isolated in England During 1995–1996 Using Polymerase Chain Reaction Restriction

J.S. Ellis,[1] C.J. Sadler,[1] P. Laidler,[1] H. Rebelo de Andrade,[2] and M.C. Zambon[1]*

[1]Enteric and Respiratory Virus Laboratory, Central Public Health Laboratory, London, United Kingdom
[2]Centro Nacional Da Gripe, Instituto Nacional de Saude, Lisbon, Portugal

A polymerase chain reaction-restriction (PCR-restriction) endonuclease assay was developed to allow rapid analysis of influenza A H3N2 viruses circulating in England during 1995–1996. Restriction endonuclease digestion with two enzymes of amplicons derived from PCR of the HA1 portion of the influenza haemagglutinin (HA) gene was able to differentiate antigenically similar influenza strains into two groups. Group I variants were similar genetically to the 1995/96 vaccine strain, A/Johannesburg/33/94, whereas the HA sequences of Group II variants were similar genetically to the reference virus A/Thessaloniki/1/95. Of the 700 England A H3N2 strains isolated between February 1995 and the end of April 1996, 384 were analysed by this method. PCR-restriction analysis of sequential influenza isolates revealed a temporal alteration in prevalence of the two variants. Groups I and II variants cocirculated with equal frequency during a period of sporadic influenza activity, but following the onset of epidemic influenza activity in 1995, only Group II variants were detected. PCR- restriction analysis was found to be a rapid method for studying genetic variation which could be applied to a large number of samples and provide information about the direction of genetic drift in the HA gene of influenza virus. ***J. Med. Virol. 51:234–241, 1997.***

KEY WORDS: influenza; haemagglutinin; restriction enzyme; molecular epidemiology

INTRODUCTION

Influenza A viruses are negative sense RNA viruses. The genome of influenza consists of eight segments of single stranded RNA which code for a minimum of ten gene products. Influenza A viruses are subtyped according to the properties of their surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA). There are currently 15 HA (H1–H15) and nine NA (N1–N9) subtypes. Influenza A subtypes which cause human disease undergo rapid and unpredictable antigenic variation. The accumulation of point mutations in the HA and NA genes, which code for the surface antigens of influenza virus, is the major cause of antigenic drift of influenza A viruses. In addition, influenza A viruses are subject to antigenic shift, which occurs when the segmented genes of different subtypes of influenza A reassort in a single host, yielding a variant with a novel pairing of surface antigens. Antigenic shift took place in 1957 and 1968 with the emergence of influenza A H2N2 and influenza A H3N2, respectively. As a consequence of antigenic drift or antigenic shift, influenza variants may not react or react poorly with protective antibodies raised to previously circulating variants, rendering the human host susceptible to reinfection. Thus, review of the antigenic composition of the influenza vaccine occurs annually, to ensure that there is the best possible match between prevailing strains and current vaccine.

Continual surveillance of the genetic diversity and antigenic profile of circulating strains is required. This is particularly important for influenza A H3N2 strains because viruses of this subtype have often been associated with excess mortality [Noble, 1982]. Since 1968, when influenza A H3N2 viruses were first identified, they have continued to circulate and show antigenic drift in different regions of the world [Cox and Bender, 1995]. Information about circulating influenza viruses and their antigenic profile is provided through national and international surveillance networks in approximately 100 countries, coordinated by the World Health Organisation (WHO). Analysis of antigenic variation of England strains is usually carried out by haemagglu-

*Correspondence to: M.C. Zambon, Enteric and Respiratory Virus Laboratory, Central Public Health Laboratory, 61, Colindale Avenue, London NW9 5HT, UK.

Accepted 18 October 1996

tination inhibition (HI) and/or neuraminidase inhibition assays and forms the basis of the information supplied annually from the United Kingdom to the WHO for review of antigenic composition of the influenza vaccine.

In recent years, sequence analysis of the HA1 domain of the HA gene, which contains the major antigenic determinants of influenza virus [Wilson et al., 1981; Wiley et al., 1981], has been used increasingly to supplement the traditional analysis of antigenic variation using ferret antisera in HI assays. Whilst sequence analysis represents a powerful tool for determining the extent and nature of genetic variation, it is expensive and time consuming and can be performed only on a small percentage of isolates. RNA-RNA hybridisation and oligonucleotide mapping, which have been used previously to address questions of genetic diversity of influenza strains [Scholtissek et al., 1978a, b; Nakajima et al., 1980], are not suitable for large-scale, rapid analysis. It would be helpful, therefore, to develop methods which allow analysis of genetic variation on a large number of strains, which could be used to indicate the direction of genetic drift in the HA molecule.

We describe the use of restriction enzymes to digest products of polymerase chain reaction (PCR) amplification derived from the HA1 portion of the HA molecule of influenza A (PCR restriction) and show how this may be used to differentiate either between antigenically similar genetic variants or between vaccine-like strains and currently circulating strains.

The application of PCR restriction to sequential influenza A H3N2 strains isolated in England in 1995–1996 demonstrated a temporal alteration in distribution of circulating variants between epidemic periods.

MATERIALS AND METHODS

Epidemiological Data

Data on the rates of "influenza and influenza like illness" were collected by the Birmingham Unit of the Royal College of General Practitioners (RCGP) and are based on weekly returns from approximately 95 practices throughout England and Wales, covering a population of 700,000–800,000 [Birmingham Research Unit, 1977]. These practices record and index all new illnesses as they occur and report every week to the Birmingham Research Unit, where they are analysed to provide incidence data, presented as new episodes per 100,000 individuals. The monitoring system operates with no imposed diagnostic criteria. A subset of the practices participating in the weekly returns service also takes part in virological monitoring, as previously described [Fleming et al., 1995], by taking swabs for virus isolation from individuals presenting with "influenza and influenza like illness."

Source of Isolates

Influenza viruses were either isolated in over 40 hospital laboratories in England, Wales, and Scotland and sent to the Central Public Health Laboratory (CPHL) for antigenic typing or derived from the community-based RCGP virological surveillance scheme described above. Briefly, combined nose and throat swabs taken by general practitioners participating in the RCGP virological surveillance scheme were sent by post and primary virus isolation undertaken at CPHL. Once the virus isolate (primary isolate from either the RCGP scheme or another laboratory) was typed as influenza A (H3N2 or H1N1) or B, it was given a unique England strain designation number in strict chronological order. England strains 1–300/95 were isolated before 1st October 1995, England strains 301–770/95 were isolated between 1 October and 31 December 1995. Strains isolated in 1996 were designated England/1/96 onwards.

Clinical Sample Inoculation

Combined nose and throat swabs in virus transport medium received in the post from the RCGP virological surveillance scheme [Fleming et al., 1995] were vortexed for 10 sec and 100 μl inoculated onto confluent Madin Darby canine kidney (MDCK) or primary rhesus monkey kidney (RMK) cells. Cells were maintained postinoculation in serum-free minimal essential medium (MEM) (Biowhittaker, Walkersville, Maryland 21793) supplemented with penicillin and streptomycin, and in the case of the MDCK cells, 1.25 μg/ml of TPCK-treated trypsin (Worthington Laboratories, Freehold, NJ) was added to the medium. The MDCK and RMK cells were incubated, rolling at either 33°C or 37°C. The medium was tested at day 7 and day 14 for haemagglutination of turkey red cells, and prior to discard a haemadsorption test was carried out using guinea pig red cells [Dowdle et al., 1979].

Virus Typing

Tissue culture fluids containing influenza viruses, either cultured directly or received from other laboratories, were typed using ferret antisera to influenza A and B prototype viruses in HI tests as described previously [Chakraverty, 1971]. Following type designation, extended antigenic analysis of influenza A H3N2 viruses was carried out using a panel of ferret antisera raised to prototype viruses circulating from 1989 onwards (Table I). All ferret antisera were treated with receptor-destroying enzyme. All HI tests were carried out using 8 haemagglutination (HA) units of virus and 0.5% v/v turkey red blood cells. A virus which showed a fourfold or less reactivity with antisera raised to earlier circulating variants was considered to show significant antigenic drift.

Viral RNA Extraction and cDNA Synthesis

Viral RNA was extracted from 50 μl of original sample or tissue culture fluid, using guanidinium thiocyanate [Boom et al., 1990]. cDNA synthesis was carried out as described previously [Ellis et al., 1995].

PCR Amplification, Sequencing, and Phylogenetic Analysis

For PCR-restriction analysis, a 590 bp region of the HA1 domain of the HA gene (nucleotides 348–938) was

TABLE I. England Influenza A H3N2 Epidemic Variants

Virus strain	Postinfection ferret antisera				Date
	A/Beijing/353/89	A/Shandong/9/93	A/Johannesburg/33/94	A/Thessaloniki/1/95	
A/Beijing/353/89	2,560	40	<10	<10	
A/Shandong/9/93	160	1,280	2,560	1,280	
A/Johannesburg/33/94	160	640	5,120	2,560	
A/Thessaloniki	320	640	2,560	5,120	
A/England/282/95	80	80	5,120	2,560	21/4/95
A/England/301/95	40	320	2,560	2,560	19/9/95
A/England/658/95	40	1,280	2,560	2,560	25/12/95
A/England/149/96	320	ND	2,560	2,560	15/1/96

Haemagglutination inhibition using postinfection ferret antisera treated with receptor-destroying enzyme. HI tests were carried out using 8 HA units of virus and 0.5% v/v turkey red blood cells. A virus which shows a fourfold or less reactivity with earlier antigens or antisera or both is considered to show significant antigenic drift. ND, not done.

amplified by nested PCR, using primers previously described [Ellis et al., 1995]. Nucleotide sequencing following amplification of the HA1 domain was performed using the Dye Deoxy Terminator method (Applied Biosystems, Foster City, CA) [Ellis et al., 1995].

Neighbour-joining phylogenetic analysis [Saitou and Nei, 1987] was performed following the alignment of sequences of HA1 using the program Megalign version 1.03 (DNASTAR Inc, Madison, Wisconsin).

Restriction Enzyme Digestion

Seventeen microlitres of the nested PCR reaction mix containing the 590 bp PCR product of amplification from the HA1 portion of the influenza A H3N2 HA molecule was added to 1 μl (10–20 units) of the restriction enzymes *Hpall* or *BstN1* (New England Biolabs, Beverly, MA) overnight at 37°C or 60°C, respectively, in 10 mM Tris/HCl (pH 7.9), 10 mM $MgCl_2$, 50 mM NaCl, and 1 mM DTT. The products of restriction enzyme digestion were visualised by ethidium bromide staining following electrophoresis on a 3% NuSieve/agarose gel (3:1) (Flowgen, Lichfield Staffs, UK).

RESULTS

Epidemiology

The clinical indices of "influenza activity" derived from the RCGP weekly returns service for "influenza and influenza like illness" [Birmingham Research Unit, 1977] for the period February–September 1995 indicated a normal background level of activity (Fig. 1). Thus, virus isolates obtained during this period represented sporadic cases of influenza in the community in England and Wales. An increase in the RCGP consultation rate/100,000 population for "influenza and influenza like illness" began in England in late September 1995 and reached moderate epidemic levels in December 1995 (Fig. 1). The clinical indices of influenza activity correlated well with the peak of influenza viruses isolated and typed by CPHL, which also occurred between November and December of 1995 (Fig. 1). Of the influenza isolates received and typed in the winter of 1995–1996 (1 October 1995–30 April 1996), 95% were influenza A H3N2 subtype. This was in contrast to the winter of 1994–1995, when influenza B predominated in the United Kingdom and only 5% of strains isolated were influenza A H3N2 (Fig. 1). A total of 700 influenza AH3N2 strains were typed between February 1995 and April 1996; the majority of these (89%) were isolated after the onset of influenza epidemic activity in October 1995. Thus, the isolates obtained in the latter half of the year represent viruses derived from an epidemic period.

Antigenic characterisation of the strains received during 1995–1996 indicated little difference between strains isolated early in 1995 and those isolated in January 1996 and no evidence of significant antigenic drift over this period of time (Table I). Antigenic analysis also indicated that circulating A/England strains were closely related to the A H3N2 vaccine strain for 1995–1996: A/Johannesburg/33/94 (A/JHB/33/94) (Table I).

Sequence Analysis

Neighbour-joining phylogenetic analysis [Saitou and Nei, 1987] of the sequence of the HA1 domain of the HA gene of 12 of 45 A H3N2 strains isolated between February and September 1995 indicated that there were two groups of variants cocirculating (Fig. 2). The two groups could be classified by comparison of their HA sequences to reference prototype viruses A/JHB/33/94 (Group I) or A/Thessaloniki/1/95 (Group II) (sequence data not shown) but were antigenically very similar. To fulfill the need to characterise genetically circulating isolates and produce information about the distribution of these two antigenically similar groups, a PCR-restriction assay was developed.

PCR Restriction

PCR amplification of HA1 of H3 HA using primers to conserved regions of HA1 was carried out as described previously [Ellis et al., 1995] and produced a 590 bp amplification product (amplicon). Two restriction enzymes, *BstN1* and *HpaII,* were selected for use in a PCR-restriction assay on the basis of their ability to cut the 590 bp PCR product amplified from HA1. *BstN1* cuts A/JHB/33/94-like (Group I) strains at one site and A/Thessaloniki/1/95-like (Group II) strains at two sites, yielding two and three fragments from the PCR amplicon, respectively (Fig. 3a). *Hpall* cuts Group I strains three times and Group II strains twice, yielding four

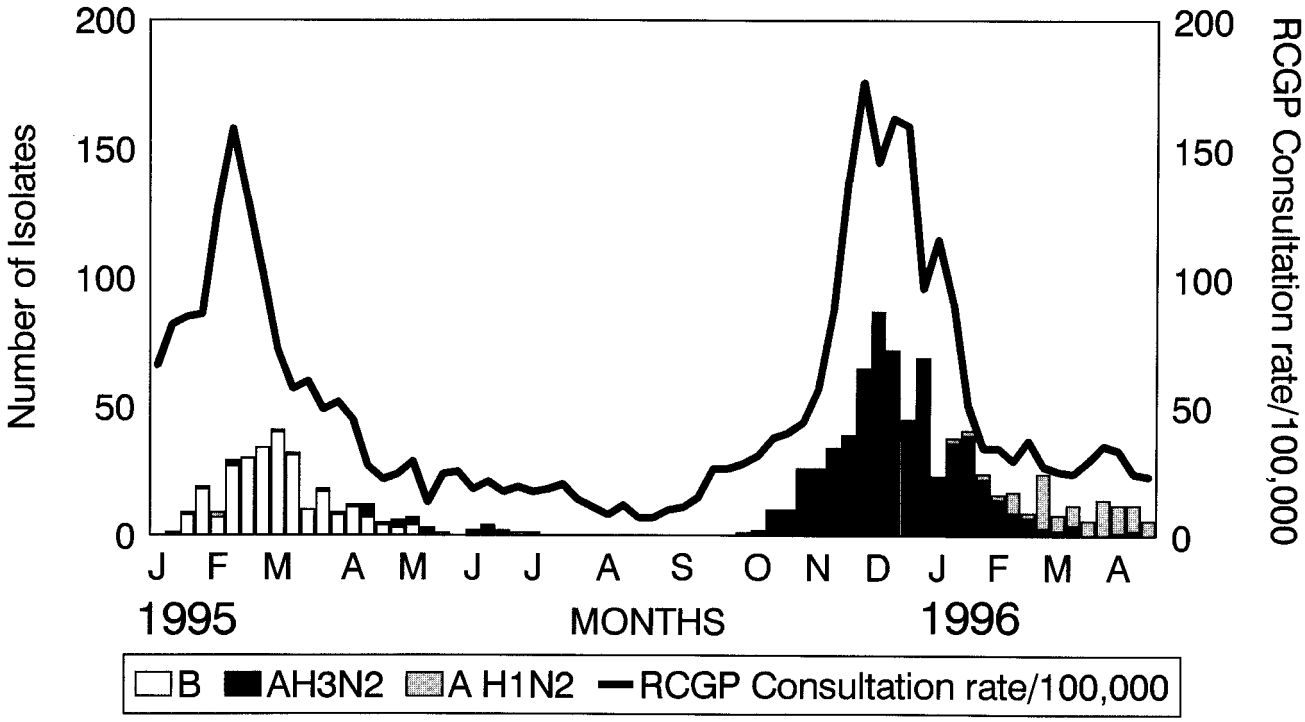

Fig. 1. The total number, type, and subtype of influenza isolates characterised by CPHL for 1995 and the first quarter of 1996 are shown in the histogram. Line represents the RCGP consultation rate/100,000 population, which is derived from the RCGP weekly returns for "influenza and influenza like illness."

and three fragments, respectively (Fig. 3b). Thus, the application of two separate enzymes to amplicons of HA1 of England strains allowed rapid differentiation of influenza strains into either the Group I or the Group II lineage, without requiring sequence analysis.

Strains were selected for restriction analysis according to England strain number designation. Sequential strains with odd numbers were analysed, and wherever possible, any isolates which had insufficient material for antigenic analysis were also included. Of the 700 England influenza A H3N2 strains isolated between February 1995 and the end of April 1996, 384 were analysed by PCR restriction with two enzymes (Table II). Of these, 60 (15.6%) could not be analysed antigenically.

PCR-restriction analysis demonstrated that two genetic variants, designated Group I and II, were cocirculating and were equally prevalent without any obvious geographical distribution (Table II) during a period of limited influenza activity in England (February–September 1995). Following the onset of epidemic influenza activity in October 1995, PCR-restriction analysis of sequential odd-numbered strains from all over England indicated the predominance of the Group II strains as only strains with Group II HA sequences were detected (Table II). The results of the PCR-restriction analysis were confirmed by sequence analysis of the HA1 of a few randomly selected isolates (Fig. 2).

Variation at Restriction Sites

Four of the 384 virus strains analysed using PCR restriction with two enzymes showed evidence of genetic variation leading to alteration in the restriction enzyme cleavage pattern. The 590 bp PCR products amplified from these influenza A H3N2 viruses were sequenced and the sequence changes determined (Table III). Three different nucleotide substitutions were observed with the enzyme *BstNI* and only one substitution with the enzyme *Hpall*. All of the nucleotide substitutions were noncoding.

PCR Restriction of Antigenic Variants of Influenza

The WHO recommended that influenza vaccines for the 1996–1997 season contain an A/Wuhan/359/95-like strain as the influenza A H3N2 component [WHO, 1996]. A/Wuhan/359/95 shows significant antigenic drift from both A/JHB/33/94 and A/Thessaloniki/1/95 viruses (Table I).

PCR-restriction analysis using the enzyme *BstN1* did not distinguish A/Wuhan/359/95 from either A/Thessaloniki/1/95- or A/JHB/33/94-like strains, but the pattern of cleavage with this enzyme correlated with the observation that A/Wuhan/359/95 was more closely related genetically to A/Thessaloniki/1/95 than to A/JHB/33/94 (Fig. 2). However, digestion of A/Wuhan/359/95 with *Hpall* did result in a difference in cleavage pattern, corresponding to the creation of an

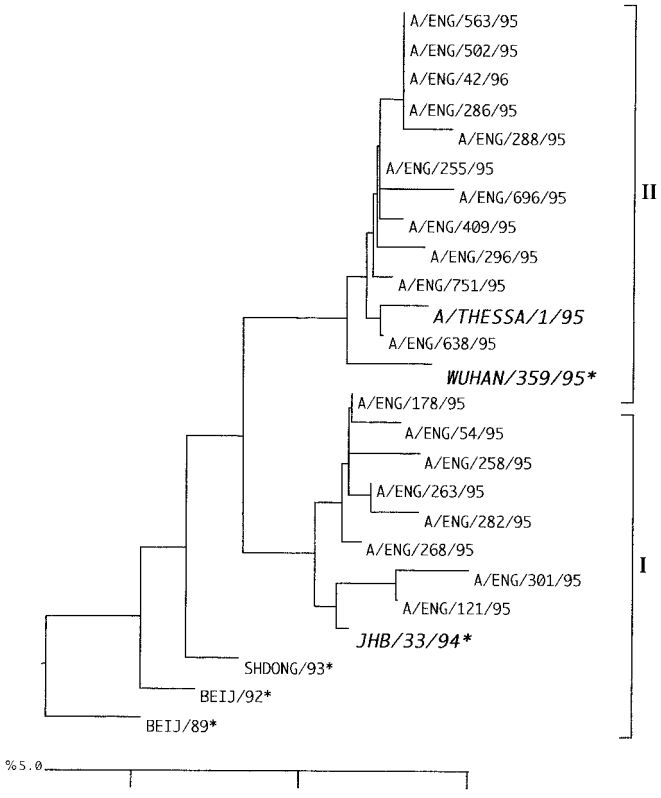

Fig. 2. Phylogenetic tree of the HA1 domains of the HA gene of influenza A H3N2 viruses, based on amino acid differences. The scale beneath the tree shows the percentage difference between sequences. Group I sequences are A/JHB/33/94-like. Group II sequences are A/Thessaloniki/1/95-like. *Denotes vaccine strains

Hpall restriction site at coding position 421 in HA1 (Fig. 4). This was due to a base substitution in A/Wuhan/359/95 which was not present in Group I or Group II variants or any of the restriction enzyme variants described earlier. The mobility difference in the fragment produced from *Hpall* restriction enzyme digestion of the 590 bp fragment could be used to screen for the presence of A/Wuhan/359/95-like viruses directly from clinical material or cultured virus material in the absence of sufficient material to perform antigenic analysis. Thus, PCR restriction could be used to identify antigenic as well as genetic variants.

Antigenic analysis of 85% of England strains received in 1995–1996 showed no evidence of strains antigenically similar to A/Wuhan/359/95. Analysis of the restriction fragment profile of sequential odd-numbered England influenza A H3N2 strains, including those showing variation at restriction cleavage sites and the 60 (15%) isolates for which antigenic analysis was not possible, also did not reveal any viruses similar genetically to A/Wuhan/359/95.

DISCUSSION

Influenza A H3N2 viruses isolated in England during the period February 1995 to the end of April 1996 were very similar antigenically in HI assays. Isolates were closely related antigenically to the 1995/96 vaccine strain, A/JHB/33/94. However, sequence analysis of the HA1 domain of HA of 12/45 strains isolated during February–September 1995, a period of sporadic influenza activity, revealed that two distinct groups of genetic variants were cocirculating. The two variants were distinguishable by comparison of their HA1 sequences to the reference viruses A/JHB/33/94 and A/Thessaloniki/1/95 [Hay, 1996]. We have classified these genetic variants as Group I and Group II, respectively. To fulfill the need for a rapid means of discriminating between Group I and Group II influenza A H3N2 strains, we developed a method based on restriction endonuclease digestion of a 590 bp PCR product amplified from the HA1 region of the HA gene. The method could be applied either directly to clinical samples or to tissue culture fluids containing influenza virus and was applied to 32 of the 45 influenza A H3N2 viruses isolated from February to September 1995 which had not been sequenced. The results showed that Group I and Group II variants were equally prevalent throughout England during a period of sporadic activity (Table II). However, similar PCR-restriction analysis of strains which were isolated following the onset of epidemic activity in 1995 showed that only Group II viruses were detected. No Group I strains were detected by PCR-restriction analysis after September 1995 (Table II). The reason for the observed change in the relative frequency of the two variants during this period of time is unknown. However, it is of some interest that, although the coding changes between Group I and Group II variants do not appear to lead to major antigenic changes detectable using polyclonal ferret antisera, they may have important biological consequences which remain undetermined. A total of 22 base changes between A/JHB/33/94 (Group I) and A/Thessaloniki/1/95 (Group II) were found in the coding region of HA1 [Hay, 1996], which constitutes a 2.1% difference between the two variants over this section of HA. Eleven of the coding changes between A/JHB/33/94 and A/Thessaloniki/1/95 result in amino acid substitutions, at positions 47, 121, 124, 137, 172, 197, 216, 219, 226, 248, and 278, all of which map to regions flanking the major antigenic sites of HA1 (Fig. 5). This probably accounts for the lack of variation in antigenic profile of the two groups when tested in HI assays with a panel of ferret antisera. Four of the coding changes were conservative and seven coding changes nonconservative. Amino acid changes at positions 216 and 219 flank the receptor binding site, and the nonconservative coding change from glutamine to isoleucine at position 226 is at the receptor binding site itself. There was also the loss of two glycosylation sites in A/Thessaloniki/1/95 compared to A/JHB/33/94 at coding positions 246 and 276 due to the coding changes at 248 and 278. Alteration in the receptor binding site or in the glycosylation of HA may affect the ability of a virus variant to replicate. The results of the differences observed on the properties of the HA molecule of Group I and Group II variants have not been investigated but

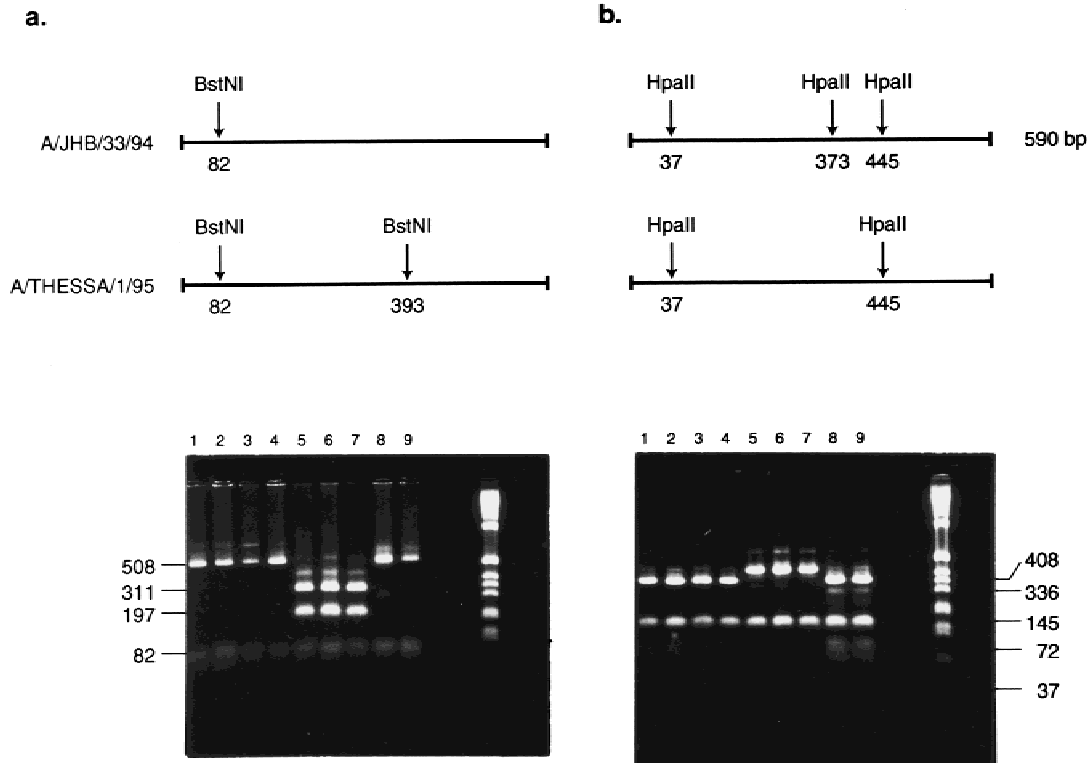

Fig. 3. PCR-restriction analysis of influenza A H3 HA using restriction endonucleases. **a:** *Bst N1.* **b:** *Hpa II.* Lanes 1a/b, A/JHB/33/94; 2a/b, A/Eng/121/95; 3a/b, A/Eng/263/95; 4a/b, A/Eng/275/95; 5a/b, A/Thessaloniki/1/95; 6a/b, A/Eng/291/95; 7a/b, A/Eng/307/95; 8a/b, A/Eng/301/95; 9a/b, A/Eng/313/95.

TABLE II. PCR-Restriction Analysis of A/England H3N2 Strains 1995–1996

Date	Total number of isolates	Number analysed	A/JHB33/94-like (Group I)	A/Thessa/1/95-like (Group II)
1/1/95–30/9/95	45	32	15	17
1/10/95–30/4/96	650	352	0	352

TABLE III. Influenza A/England H3N2 PCR-Restriction Variants

Isolate	HA[a] sequence	Restriction enzyme	Site	Nucleotide[b] position	Amino acid position
A/Eng/241/95	Group I	*HpaII*	Lost	386 ccg → cca	103 Proline
A/Eng/462/95	Group II	*BstNI*	Lost	429 ttg → ctg	118 Leucine
A/Eng/601/95	Group II	*BstNI*	Gained	470 gct → gcc	131 Alanine
A/Eng/122/96	Group II	*BstNI*	Lost	428 acc → aca	117 Threonine

[a]Sequences are classified compared to the reference strains A/JHB/33/94 (Group I) or A/Thessaloniki/1/95 (Group II).
[b]Nucleotide positions are numbered with respect to the HA vRNA.

may help to explain why Group II variants appear to have replaced Group I variants in England.

It has been observed that for influenza A viruses genetically distinct lineages cocirculate for significant periods of time [Cox and Bender, 1995] and that individual influenza genes generally accumulate mutations at an approximately constant rate [Hayashida et al., 1985]. It is therefore important to know which strains are circulating and the direction of genetic drift when considering candidate strains for vaccine selection. Analysis of A/England influenza H3N2 isolates using the PCR-restriction assay was able to differentiate rapidly between different genetic variants which were similar antigenically and, when used on sequential isolates, provided information about the direction of genetic drift. The results of PCR-restriction analysis show that influenza A H3N2 strains isolated in England during the epidemic period 1995–1996 are evolving along the lineage represented by A/Thessaloniki/1/95.

PCR restriction can also be used to differentiate vaccine-like strains from other circulating variants, which

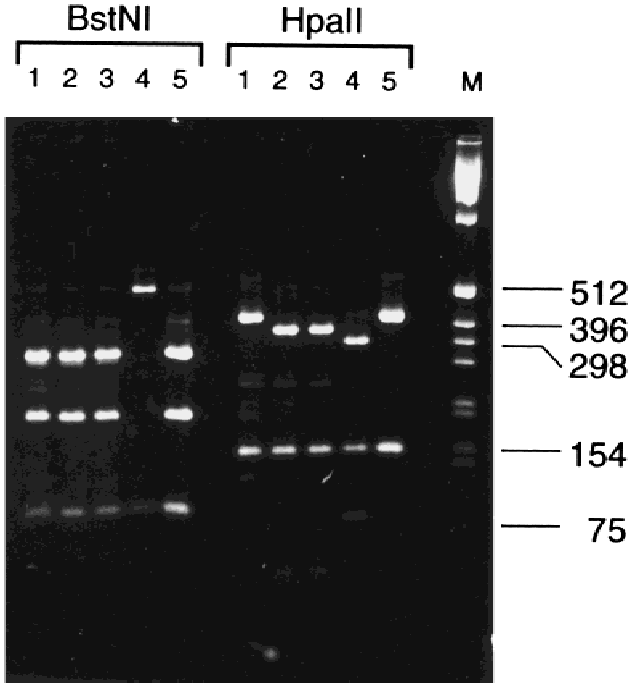

Fig. 4. PCR-restriction analysis of A/Wuhan/359/95. Lanes 1, A/Thessaloniki/1/95; 2, A/Wuhan/359/95; 3, A/Nanchang/933/95 (A/Wuhan/359/95-like); 4, A/JHB/33/94; 5, A/England/288/95 (A/Thessaloniki/1/95-like). Products of PCR amplification (590 bp) were digested with either *BstN1* or *HpaII*. M, DNA molecular weight markers.

may be useful in periods when antigenically different variants are cocirculating, but there is difficulty in growing virus isolates to provide sufficient material for antigenic analysis. A/Wuhan/359/95 was isolated in China in July 1995, and A/Wuhan/359/95-like viruses are antigenically distinguishable in HI assays from both A/JHB/33/94 and A/Thessaloniki/1/95 (Table I). A/Wuhan/359/95-like viruses have spread to Hong Kong, Singapore, Japan, the United States, Australasia, and Europe since July 1995 [WHO, 1996]. Although A/Wuhan/359/95-like variants account currently for a minority of influenza A H3N2 isolates worldwide, antisera raised to A/JHB/33/94 or A/Thessaloniki/1/95 are only poorly reactive in HI tests with these viruses, which suggests that an influenza vaccine containing an H3N2 strain similar antigenically to A/JHB/33/94 would provide poor protection against A/Wuhan/359/95-like viruses [WHO, 1996]. This information in conjunction with information about the spread of A/Wuhan/359/95-like viruses signalled a need to update the composition of the influenza vaccine for 1996–1997 to include A/Wuhan/359/95-like strains [WHO, 1996].

Sequence and phylogenetic analyses of the HA1 of A/Wuhan/359/95 indicated that it was much more closely related to A/Thessaloniki/1/95 than to A/JHB/33/94 (Fig. 2). Analysis of the nucleotide sequence of A/Wuhan/359/95 showed that there were eight noncoding changes and six coding changes at residues 135, 136, 145, 194, 248, and 272 in the HA1 region between

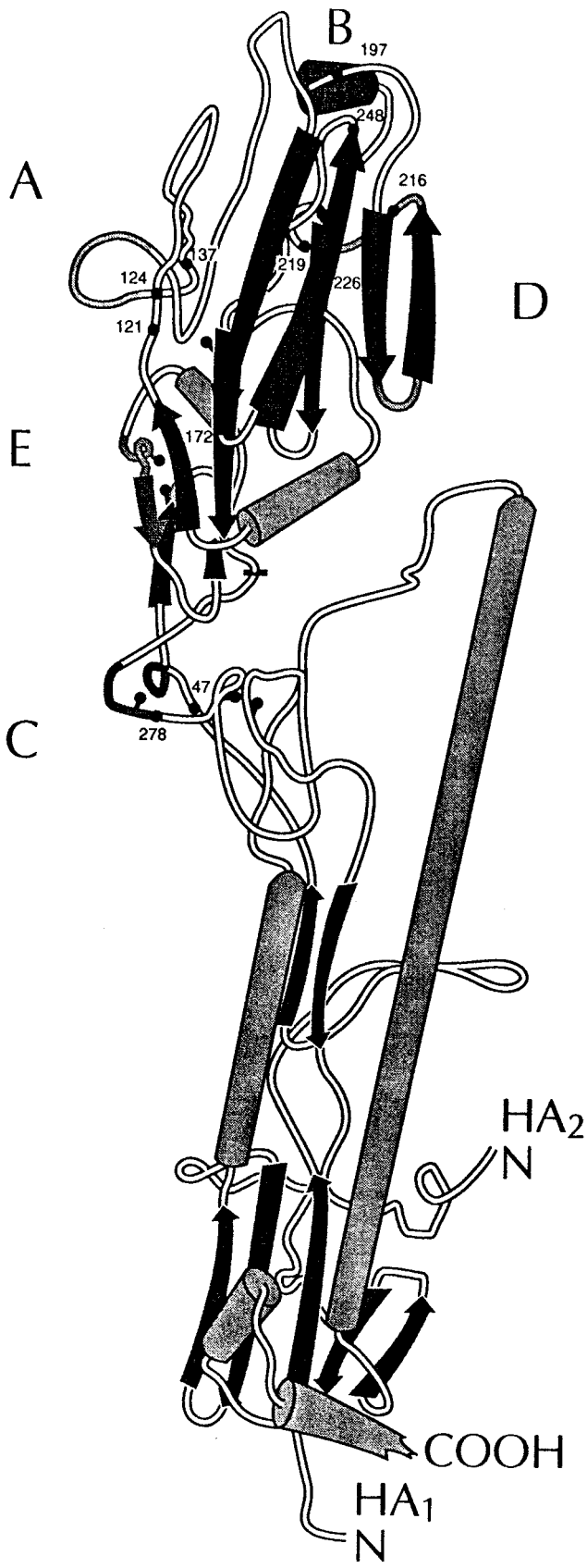

Fig. 5. Location of amino acid substitutions in the HA between A/JHB/33/94 and A/Thessaloniki/1/95. **A–E** are antigenic sites described by Wiley *et al.* [1981]. Drawing of HA monomer is based on the HA monomer of A/Hong Kong/68 (H3N2).

A/Wuhan/359/95 and A/Thessaloniki/1/95. At least four of the coding changes found in A/Wuhan/359/95 map to the major antigenic sites of HA1, which probably accounts for the major changes in antigenicity of this variant (data not shown). The PCR-restriction assay described in this study can detect rapidly A/Wuhan/359/95-like strains since the restriction enzyme *Hpall* can distinguish A/Wuhan/359/95 from A/Thessaloniki/1/95 and A/JHB/33/94 viruses. However, the results of this study have shown that there is no evidence for the circulation of A/Wuhan/359/95-like viruses in England during 1995–1996 to date. PCR-restriction analysis of influenza variants, as described here, can be performed directly on clinical material and yield accurate information about the genetic profile of influenza isolates. This is useful when rapid genetic analysis of outbreaks of influenza is required, as it often is at the beginning of epidemic activity, and there is public health interest in determining the similarity of circulating viruses to a vaccine formulated annually.

In conclusion, the PCR-restriction assay described combines the high specificity of restriction analysis with the high sensitivity of PCR. The method is rapid and simple to perform and is particularly useful for analysing strains which cannot be subjected to antigenic analysis due to insufficient material. PCR-restriction analysis in combination with antigenic analysis provides a valuable asset for the continued surveillance of circulating influenza viruses.

ACKNOWLEDGMENTS

We acknowledge the excellent technical assistance of Karen Isaacs. We thank Dr Alan Hay, National Institute for Medical Research, Mill Hill, London, for the gift of A/Thessaloniki/1/95 virus and Dr Nancy Cox, Influenza Branch, CDC, Atlanta, GA, for the sequence of A/Wuhan/359/95. We also thank Mr Jon White, Medical Illustration, Central Public Health Laboratory, Colindale, for the drawing of the HA monomer based on that by Hidde Ploegh.

REFERENCES

Birmingham Research Unit, Royal College of General Practitioners (1977): Influenza. Journal of the Royal College of General Practitioners 27:544–551.

Boom RS, Sol CJA, Salimans MMM, Jansenel Werhteim-van Dillen PME, Van Der Noovdaa J (1990): Rapid and simple method for purification of nucleic acids. Journal of Clinical Microbiology 28: 493–503.

CDWR (1995): Influenza A: A school outbreak to start the term. Communicable Disease Weekly Report 5:39.

Chakraverty P (1971): Antigenic relationships between influenza B viruses. Bulletin of the World Health Organization 45:755–766.

Cox NJ, Bender CA (1995): The molecular epidemiology of influenza viruses. Seminars in Virology 6:359–370.

Dowdle W, Kendal AP, Noble GR (1979): Influenza viruses. In Lennette EH and Schmidt NJ (eds): "Diagnostic Procedures for Viral Rickettsial and Chlamydiae Infections." 5th ed. pp 585–609.

Ellis JS, Chakraverty P, Clewley JP (1995): Genetic and antigenic variation in the haemagglutinin of recently circulating human influenza A(H3N2) viruses in the United Kingdom. Archives of Virology 140:1889–1904.

Fleming DM, Chakraverty P, Sadler C, Litton P (1995): Combined clinical and virological surveillance of influenza in winters of 1992 and 1993. British Medical Journal 311:290–291.

Hay AJ (1996): "Annual Report of the WHO International Collaborating Centre for Reference and Research on the Influenza Virus for the Period August 1995 to July 1996." London: National Institute for Medical Research.

Hayashida H, Toh H, Kikuno R, Miyata T (1985): Evolution of influenza virus genes. Molecular Biology and Evolution 2:289–303.

Nakajima S, Nakajima K, Takeuchi Y, Sugiura A (1980): Influenza surveillance based on oligonucleotide mapping of RNA of H1N1 virus prevalent in Japan 1978–1979. Journal of Infectious Diseases 142:492–502.

Noble GR (1982): Epidemiological and clinical aspects of influenza. In Beare AS (ed): "Basic and Applied Influenza Research." FL: CRC Press, pp 11–50.

Saitou N, Nei M (1987): The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular and Biological Evolution 4:406–425.

Scholtissek C, Rohde W, von Hoyningen V, Rott R (1978a): On the origin of the human influenza subtype H2N2 and H3N2. Virology 87:13–20.

Scholtissek C, von Hoyningen V, Rott R (1978b): Genetic relatedness between the new 1977 epidemic strains (H1N1) of influenza and human influenza strains isolated between 1947 and 1957 (H1N1). Virology 87:613–617.

WHO (1996): Recommended composition of influenza virus vaccines for use in the 1996–1997 season. Weekly Epidemiological Record 71:57–64.

Wiley DC, Wilson IA, Skehel JJ (1981): Structural identification of the antibody-binding sites of Hong Kong influenza haemagglutinin and their involvement in antigenic variation. Nature 289:373–378.

Wilson IA, Skehel JJ, Wiley DC (1981): Structure of the haemagglutinin membrane glycoprotein of influenza virus at 3A resolution. Nature 289:366–373.